REMARKS

Claims 1 and 3 have been amended without prejudice. Support for the amendments can be found, e.g., on page 5, first full paragraph of the specification as filed and in original claims 1 and 3.

Claims 9-14 and 19-20 were previously canceled without prejudice in response to the Restriction Requirement.

Claims 1-8 and 15-18 are pending.

Applicants submit that no new matter has been added by virtue of this amendment.

Indefiniteness Rejections

Claims 1-8 and 15-18 were rejected under 35 U.S.C. 112, second paragraph. The Examiner stated that "claim 1 does not contain a positive process step that clearly relates back to the preamble." See Office Action, page 2.

In response, Applicants respectfully note that claim 1 has been amended without prejudice to recite:

> 1. A method for diagnosing cancer, said method comprising detecting a soluble GPC3 protein level in a test sample, and determining whether said detected soluble GPC3 level is greater than a basal level of GPC3.

Applicants respectfully submit that the indefiniteness rejection of claim 1 has been overcome by the amendments to claim 1.

With regard to claim 3, the Examiner stated "[c]laim 3 is indefinite because it is not clear whether the N-terminal peptide of GPC3 that is being detected is a fragment consisting of the 1st amino acid to the 374th amino acid or is any fragment contained within the fragment consisting of the 1st amino acid to the 374th amino acid." See, page 2 of the Office Action.

In response, Applicants respectfully note that claim 3 has been amended without prejudice to recite:

> 3. The method for diagnosing cancer of claim 2, wherein the N-terminal peptide of GPC3 is a peptide fragment having the amino acid sequence of GPC3 consisting of the 1st amino acid to the 358th amino acid.

Applicants submit that amended claim 3 makes it clear that the N-terminal peptide of GPC3 that is being detected is "is a peptide fragment having the amino acid sequence of GPC3 consisting of the 1st amino acid to the 358th amino acid."

For the foregoing reasons, Applicants respectfully request withdrawal of the indefiniteness rejections.

Enablement Rejections

Claims 1-8 and 15-18 were rejected under 35 U.S.C. § 112, first paragraph. The Examiner stated that "[t]he teaching of the specification cannot be reasonably extrapolated to enable the invention because one of skill in the art could not predict that the invention would function as contemplated given that the GPC3 protein was not demonstrated to be overexpressed in any in vivo cancer, particularly in any in vivo human cancer." See page 5 of the Office Action.

In response, Applicants submit that one skilled in the art would be able to practice the invention based on the present specification without any additional guidance and without any undue experimentation, at the very least, for the following reasons.

Applicants respectfully direct the Examiner's attention to Example 3 of the present specification. Example 3 describes the experiment, wherein "[t]he soluble GPC3 was detected [in vivo] ... in the sera of the mice," 53 days after HepG2 human hepatic cancer cells

had been grafted into the mice (when tumor mass had been successfully formed.) See, pages 42-44 of the present specification as filed. The concentration of purified soluble GPC3 in the mice with tumors was 23 to 90 ng/ml, in contrast to the control mouse sera, wherein the level was "below the detection limit." See, original specification as filed, page 44. Accordingly, Applicants submit that the specification, at the very least, does demonstrate that there is a correlation between a level of soluble GPC3 in sera and the presence of in vivo tumor.

In response to the Examiner's statement that "the prior art is replete with examples in which expression levels of mRNA are not correlated with expression levels of the encoded protein, Applicants direct the Examiner's attention to (i) Celis et al. (FEBS Lett. 2000 Aug 25;480(1):2-16, *"Gene expression profiling: monitoring transcription and translation products using DNA microarrays and proteomics"*), listed as document AH on the attached Form PTO-1449; and (ii) Anderson et al. (Electrophoresis. 1997 Mar-Apr;18(3-4):533-7, *"A comparison of selected mRNA and protein abundances in human liver"*), listed as document AI on that attached Form PTO-1449.

Applicants submit that the Celis document describes in part that the differences in gene expression are reflected in the differences in protein expression, despite the fact that some messengers are transcribed but not translated. See, e.g, p.6, 3. Proteomics, first paragraph.

Applicants submit that the Anderson document describes in part that moderate correlation (r=0.48) was found between mRNA and corresponding protein abundance, and that this level of correlation may be attributed to degradation of transcribed mRNA and degradation of translated protein. See, p.536, 4. Discussion, lines 13-22).

Applicants submit that these documents demonstrate, e.g., that while expression of mRNA does not always mean expression of protein, it was well established in the art at the priority date of the present application that there is a correlation between mRNA expression and protein expression. In fact, Applicants submit that, *inter alia* because of this correlation,

numerous researches comparing mRNA expression levels in cancerous and normal tissues were carried before the priority date of the present application to screen for molecules which are highly expressed specifically in cancerous tissues. See e.g., Liotta et al., NATURE REVIEWS GENETICS Volume 1, 48-56, 2000, "*Molecular Profiling of Human Cancer*", listed as document AJ on the attached Form PTO-1449; see also Bertucci et al.. THE LANCET ONCOLOGY Vol. 2, 674-82, 2001, *"Gene expression profiling of cancer by use of DNA arrays: how far from the clinic?"*, listed as document AK on the attached Form PTO-1449; see also Nelson et al., Journal of the National Cancer Institute, Vol. Sa, No.24, 180305, 1996, *"Microarrays Pave the Way to 21st Century Medicine"*, listed as document AL on the attached Form PTO-1449; see also Hough et al., Cancer Res. 60, 6281-87, 2000, *"Large-Scale Serial Analysis of Gene Expression Reveals Genes Differentially Expressed in Ovarian Cancer"*, listed as document AM on the attached Form PTO-1449; see also Zhow et al., Cancer Epidemiology, Biomarkers & Prevention Vol. 7, 109-12, 1998, "*Identifying Markers for Pancreatic Cancer by Gene Expression Analysis*", listed as document AN on the attached Form PTO-1449.

Applicants further submit that, in view of the teachings of above-mentioned documents and the teachings of the present specification (e.g., that GPC3 is highly expressed in cancerous tissues but poorly expressed in normal tissues, and that GPC3 is expressed at protein level in HepG2 xenograft and is detected in the serum), a person skilled in the art would reasonably expect, contrary to the Examiner's assertion, e.g., that most of the GPC3 mRNA would be expressed as a protein and would not undergo degradation. Accordingly, Applicants submit that at the priority date of the present application, one skilled in the art would recognize that a molecule specifically expressed in cancerous tissue at mRNA level (i.e., as described in the present specification) would likely be highly expressed in that tissue at protein level.

Applicants further submit that, although various properties may be different in cell lines and tumor tissues in vivo other than HepG2, at the priority date of the present application, it was known that HepG2 demonstrates similar properties in xenograft-bearing

mice as those observed in human hepatic cancer in vivo. See, e.g., Huber et al. (Cancer Res. 45, 4322-29, 1985, *"Tumorigenicity and Transcriptional Modulation of c-myc and N-ras Oncogenes in a Human Hepatoma Cell Line"* (listed as document AP on the attached Form PTO-1449) , describing *inter alia* that hepatic markers AFP and GGT were detected in HepG2 cells (p.4325, Discussion, first paragraph); See also Seow TK. et al. (Proteomics 1, 1249-1263, 2000, *"Hepatocellular carcinoma: From bedside to proteomics."* (listed as document AO on the attached Form PTO-1449), describing *inter alia* that HepG2, Huh6 and PLC/PRF/5 cell lines, which are derived from hepatic cancer and produce AFP, show similar expression profile from each other, and that such an expression pattern is different from cell lines derived from hepatic cancer which do not produce AFP and from tumor cell lines derived from cancers other than hepatic cancer (p.12583, 2.1.4 cDNA microarray analysis of hepatoma cell lines, first paragraph); See also Knowles BB. Et al. (Science 209, 497-99,1980, *"Human Hepatocellular Carcinoma Cell Lines Secrete the Major Plasma Proteins and Hepatitis B Surface Antigen"*), listed as reference AT on the attached Form PTO-1449, which describes *inter alia* that HepG2 and Hep3B cell lines retain the ability of secreting serum proteins characteristic of parenchymal hepatocyte.

Applicants submit that the teachings of the above-mentioned documents support Applicants position that at the priority date of the present application one skilled in the art would consider that HepG2 cells will retain the properties of hepatic cancer in vivo.

In addition, Applicants submit that HepG2 is a cell line broadly used in the art for investigating functions of hepatic cancer. See, e.g., Kraft et al., Cancer Res. 53, 652-57, 1993, *"Suramin Inhibits Growth and Yet Promotes Insulin-like Growth Factor II Expression in HepG2 Cells"*, listed as document AQ on the attached Form PTO-1449; See also, Castaneda et al., J Cancer Res Clin Oncol, 126, 503-10, 2000, *"Cytotoxicity of millimolar concentrations of ethanol on HepG2 human tumor cell line compared to normal rat hepatocytes in vitro"*; listed as document AR on the attached Form PTO-1449; See also, Pati et al., Endocrinology, 136, 1,75-84, 1995, *"Inhibition of Human Hepatocarcinoma Cell Proliferation by Mammalian and Fish Gonadotropin-Releasing Hormons"*, listed as document AS on the attached Form

PTO-1449; See also U.S. Patent No. 5,644,026; U.S. Patent No. 5,760,000; U.S. Patent No. 5,807,860; U.S. Patent No. 5,843,937; U.S. Patent No. 6,268,336; and U.S. Patent No. 6,486,144.

Applicants submit that the teachings of the above-mentioned documents support Applicants position that that one skilled in the art would expect HepG2 to exhibit similar properties as human hepatic cancer in vivo, e.g., because that HepG2 xenograft mice have been widely used as a model of human hepatic cancer.

Furthermore, Applicants submit herewith Appendix A, which contains experimental data which demonstrates, e.g., that overexpression of soluble GPC3 is frequently detected in human patients with hepatic cancer.

For the foregoing reasons, Applicants submit that one skilled in the art is able to practice the presently claimed methods without any additional guidance and undue experimentation, based on the present specification and the general knowledge in the art at the priority date of the present application. Therefore, Applicants respectfully request withdrawal of the enablement rejection of claims 1-8 and 15-18.

In point 7 of the Office Action, the Examiner stated that "[i]f rejection of claims 1-8 and 15-18, under 35 U.S.C. 112, first paragraph, for lack of enablement, as stated in paragraph 6 above, is overcome, claims 1-8 and 15-18 would still be rejected under 35 U.S.C. 112, first paragraph, for lack of enablement, because ... [one skilled in the art] could not predictably distinguish cancer from normal controls or patients with liver cirrhosis in the absence of a cut-off point that would differentiate between the cancer and levels of GPC3 protein found in normal patients and patients with other diseases that present with soluble GPC3 protein ..." See pages 6 and 7 of the Office Action.

As discussed above, claim 1 has been amended without prejudice to recite:

> 1. A method for diagnosing cancer, said method comprising detecting a soluble GPC3 protein level in a test sample, and determining whether said detected soluble GPC3 level is greater than a basal level of GPC3.

Applicants submit that the rejection is overcome by the amendments to claim 1.

Claims 5 and 16-17 were rejected under 35 U.S.C. § 112, first paragraph. The Examiner stated that "the detection of soluble GPC3 in blood could indicate the presence of melanoma or hepatocellular carcinoma and merely the detection of GPC3 in sera would not allow one of skill in the art to diagnose the presence of a particular cancer." In support of her position, the Examiner relied on a reference (Nakatsura et al., 2005, Biodrugs 19(2):71-77) which was published after the priority date of the present application (September 4, 2002).

In response, Applicants submit that it is inappropriate to use the Nakatsura reference, which published after the present application was filed, to support the enablement rejection of the present application, as the Nakatsura reference was not part of the art at the effective filing date of the present application[a], and that the MPEP states that "[a]ny analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in pertinent art to make and use the claimed invention." See MPEP, section 2164.01 (emphasis added).

For the foregoing reasons, Applicants respectfully request withdrawal of the rejection.

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[a] This application was filed March 4, 2005, as National Phase of International Application No. PCT/JP2003/0011320, filed September 4, 2003, which claims priority to International Application No. PCT/JP02/08997, filed September 4, 2002.

Claims 3 and 15-16 were rejected under 35 U.S.C. § 112, first paragraph. The Examiner stated that the present specification is enabling for "a method for diagnosing cancer, which comprises detecting a soluble GPC3 protein in a test sample, wherein the soluble GPC3 protein is an N-terminal peptide of GPC3 consisting of the 1st amino acid to the 358th amino acid ...." See e.g.., page 9 of the Office Action.

As discussed above, claim 3 have been amendment without prejudice to recite:

> 3. The method for diagnosing cancer of claim 2, wherein the N-terminal peptide of GPC3 is a peptide fragment having the amino acid sequence of GPC3 consisting of the 1st amino acid to the 358th amino acid.

Applicants submit that amendments to claim 3 overcomes the enablement rejection, as the Examiner acknowledged that the specification is "enabling for a method for diagnosing cancer, which comprises detecting a soluble GPC3 protein in a test sample, wherein the soluble GPC3 protein is a terminal peptide of GPC3 consisting of the 1st amino acid to the 358th amino acid...". See e.g., page 9 of the Office Action.

For the foregoing reasons, withdrawal of the enablement rejections is respectfully requested.

CONCLUSION

An early and favorable action on the merits is earnestly solicited. The Examiner is respectfully requested to contact the undersigned in the event that a telephonic interview will advance the prosecution of this application.

Respectfully submitted,
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APPENDIX A

		A450
Normal human serum	1	0.047
	2	0.045
	3	0.058
	4	0.043
	5	0.047
	6	0.045
	7	0.051
	8	0.039
	9	0.049
	10	0.045
	11	0.043
	12	0.100
	13	0.057
	14	0.045
	15	0.056
	16	0.043
	17	0.044
	18	0.066
	19	0.039
	20	0.047
	21	0.036
	22	0.048
	23	0.041
	24	0.039
	25	0.039
	26	0.041
	27	0.036
	28	0.059
	29	0.076
	30	0.152
	31	0.052
	32	0.039
	33	0.136
	34	0.066
	35	0.052
	36	0.045
	37	0.052
	38	0.049
	39	0.049
	40	0.048
	41	0.038
	42	0.054
	43	0.043
	44	0.054
	45	0.039
	46	0.038
	47	0.056
	48	0.056
	49	0.035
	50	0.032
	51	0.043
	52	0.041
	53	0.056
	54	0.046
	55	0.037
	56	0.037
	57	0.042
	58	0.044
	59	0.040
	60	0.030
	61	0.046
	62	0.047
	63	0.060
	64	0.052
	65	0.045
	66	0.062
	67	0.054
	68	0.039
	69	0.070
	70	0.043
	71	0.056
	72	0.086
	73	0.045
	74	0.049
	75	0.036
	76	0.066
	77	0.037
	78	0.045
	79	0.060
	80	0.054
Mean		0.051
SD		0.019
Mean+3SD		0.108

tentative cut off = mean + 3SD

	Sample No.	Determination	A450	Sample No.	Determination	A450
HCC patients serum	1	Positive	1.818	87	Positive	0.119
	2	Positive	1.431	88	Positive	0.118
	3	Positive	1.164	89	Positive	0.117
	4	Positive	0.99	90	Positive	0.117
	5	Positive	0.861	91	Positive	0.117
	6	Positive	0.756	92	Positive	0.117
	7	Positive	0.710	93	Positive	0.116
	8	Positive	0.616	94	Positive	0.115
	9	Positive	0.614	95	Positive	0.113
	10	Positive	0.574	96	Positive	0.113
	11	Positive	0.531	97	Positive	0.111
	12	Positive	0.491	98	Positive	0.11
	13	Positive	0.491	99	Positive	0.108
	14	Positive	0.486	100	Positive	0.108
	15	Positive	0.465	101	Positive	0.108
	16	Positive	0.458	102	Positive	0.108
	17	Positive	0.406	103	Negative	0.106
	18	Positive	0.376	104	Negative	0.105
	19	Positive	0.373	105	Negative	0.104
	20	Positive	0.372	106	Negative	0.102
	21	Positive	0.358	107	Negative	0.099
	22	Positive	0.345	108	Negative	0.096
	23	Positive	0.344	109	Negative	0.095
	24	Positive	0.330	110	Negative	0.095
	25	Positive	0.319	111	Negative	0.094
	26	Positive	0.303	112	Negative	0.094
	27	Positive	0.300	113	Negative	0.093
	28	Positive	0.292	114	Negative	0.092
	29	Positive	0.285	115	Negative	0.092
	30	Positive	0.275	116	Negative	0.092
	31	Positive	0.270	117	Negative	0.091
	32	Positive	0.265	118	Negative	0.091
	33	Positive	0.25	119	Negative	0.089
	34	Positive	0.249	120	Negative	0.089
	35	Positive	0.24	121	Negative	0.088
	36	Positive	0.236	122	Negative	0.087
	37	Positive	0.235	123	Negative	0.087
	38	Positive	0.235	124	Negative	0.086
	39	Positive	0.234	125	Negative	0.086
	40	Positive	0.232	126	Negative	0.085
	41	Positive	0.23	127	Negative	0.085
	42	Positive	0.227	128	Negative	0.084
	43	Positive	0.225	129	Negative	0.083
	44	Positive	0.224	130	Negative	0.082
	45	Positive	0.224	131	Negative	0.082
	46	Positive	0.221	132	Negative	0.081
	47	Positive	0.218	133	Negative	0.08
	48	Positive	0.217	134	Negative	0.08
	49	Positive	0.213	135	Negative	0.079
	50	Positive	0.209	136	Negative	0.078
	51	Positive	0.209	137	Negative	0.078
	52	Positive	0.203	138	Negative	0.077
	53	Positive	0.203	139	Negative	0.077
	54	Positive	0.203	140	Negative	0.076
	55	Positive	0.195	141	Negative	0.076
	56	Positive	0.194	142	Negative	0.075
	57	Positive	0.189	143	Negative	0.074
	58	Positive	0.188	144	Negative	0.074
	59	Positive	0.178	145	Negative	0.072
	60	Positive	0.178	146	Negative	0.072
	61	Positive	0.177	147	Negative	0.07
	62	Positive	0.177	148	Negative	0.07
	63	Positive	0.177	149	Negative	0.069
	64	Positive	0.176	150	Negative	0.069
	65	Positive	0.173	151	Negative	0.069
	66	Positive	0.158	152	Negative	0.065
	67	Positive	0.155	153	Negative	0.065
	68	Positive	0.153	154	Negative	0.062
	69	Positive	0.153	155	Negative	0.062
	70	Positive	0.15	156	Negative	0.061
	71	Positive	0.147	157	Negative	0.061
	72	Positive	0.145	158	Negative	0.06
	73	Positive	0.143	159	Negative	0.059
	74	Positive	0.138	160	Negative	0.058
	75	Positive	0.137	161	Negative	0.057
	76	Positive	0.135	162	Negative	0.057
	77	Positive	0.132	163	Negative	0.057
	78	Positive	0.131	164	Negative	0.055
	79	Positive	0.126	165	Negative	0.055
	80	Positive	0.126	166	Negative	0.053
	81	Positive	0.125	167	Negative	0.053
	82	Positive	0.125	168	Negative	0.051
	83	Positive	0.122	169	Negative	0.050
	84	Positive	0.122	170	Negative	0.049
	85	Positive	0.12	171	Negative	0.048
	86	Positive	0.12	172	Negative	0.048

HCC : hepatocarcinoma